

**CLAIMS**

1. Biologically active isolated Tat protein, fragments thereof and/or mutants and/or Tat DNA for use as a vaccine, said Tat, at picomolar to nanomolar concentrations being capable of: (i) entering and localizing in the nuclei of  
5 activated endothelial cells or dendritic cells; and/or (ii) activating the proliferation, migration and invasion of Kaposi's sarcoma (KS) cells and cytokine-activated endothelial cells protein.
2. Biologically active isolated Tat protein, fragments thereof and/or mutants and/or DNA Tat according to claim 1 further capable of: (iii) activating virus  
10 replication when added to infected cells as measured a) by the rescue of Tat-defective proviruses in HLM-1 cells after the addition of exogenous protein; and/or b) by the transactivation of HIV-1 gene expression in cells transfected with a HIV-1 promoter-reporter plasmid.
3. Biologically active isolated Tat protein, fragments thereof and/or mutants  
15 and/or Tat DNA according to claim 2 further capable of: (iv) inducing in mice the development of KS-like lesions in the presence of angiogenic factors or inflammatory cytokines.
4. Biologically active isolated Tat protein, fragments thereof and/or mutants and/or Tat DNA according to claims 1-3 at amounts ranging between 10  
20 ng/ml or less to 1 µg/ml.
5. Biologically active isolated Tat protein, fragments thereof and/or mutants and/or Tat DNA according to claims 1-4 for use in the prophylactic and/or therapeutic treatment of AIDS, tumors, syndromes and symptoms associated with HIV infection.
- 25 6. Protein or peptide or DNA vaccine, prophylactic and/or therapeutic, against AIDS, tumors, syndromes and symptoms associated with the HIV infection, comprising biologically active Tat and/or its mutants and/or portion of the protein or peptides or a DNA as defined in claims 1-4.
- 30 7. Vaccine according to claim 6 in which Tat has the following nucleotide sequence (Seq.1) :  
5'ATGGAGCCAGTAGATCCTAGACTAGAGCCCTGGAAGCATCCAGGAAGTC

AGCCTAAAACTGCTTGTACCAATTGCTATTGTAAAAAGTGTTGCTTTCATTG  
 CCAAGTTTGTTCATAACAAAAGCCTTAGGCATCTCCTATGGCAGGAAGAA  
 GCGGAGACAGCGACGAAGACCTCCTCAAGGCAGTCAGACTCATCAAGTTT  
 CTCTATCAAAGCAGCCACCTCCCAATCCCGAGGGGACCCGACAGGCC  
 5 GAAGGAATAG 3'

and any other Tat variant of any HIV type and subtype.

8. Vaccine according to claim 6 in which Tat has the following amino acid sequence :

NH<sub>2</sub>-EPVDPRLEPWKHPGSQPKTACTNCYCKKCCFHCQVCFITKAISY  
 10 GRKKRRQRRRPPQGSQTHQVLSKQPTSQSRGDPTGPKE-COOH

and any other Tat variant of any HIV type and subtype.

9. Vaccine according to claim 6 in which mutants are selected among the ones having the following nucleotide sequences or part of them:

Nucleotide sequence of cys22 mutant (Seq.2)

15 5'ATGGAGCCAGTAGATCCTAGACTAGAGCCCTGGAAGCATCCAGGAAGT  
 CAGCCTAAAACTGCGGTACCAATTGCTATTGTAAAAAGTGTTGCTTTCATT  
 GCCAAGTTTGTTCATAACAAAAGCCTTAGGCATCTCCTATGGCAGGAAG  
 AAGCGGAGACAGCGACGAAGACCTCCTCAAGGCAGTCAGACTCATCAAG  
 TTTCTCTATCAAAGCAGCCACCTCCCAATCCCGAGGGGACCCGACAGG  
 20 CCCGAAGGAATAG 3'

Nucleotide sequence of lys41 (Seq.3)

5'ATGGAGCCAGTAGATCCTAGACTAGAGCCCTGGAAGCATCCAGGAAGT  
 CAGCCTAAAACTGCTTGTACCAATTGCTATTGTAAAAAGTGTTGCTTTCAT  
 TGCCAAGTTTGTTCATAACAAACGCCTTAGGCATCTCCTATGGCAGGAA  
 25 GAAGCGGAGACAGCGACGAAGACCTCCTCAAGGCAGTCAGACTCATCAA  
 GTTTCTCTATCAAAGCAGCCACCTCCCAATCCCGAGGGGACCCGACAG  
 GCCCGAAGGAATAG 3'

Nucleotide sequence of RGDΔ mutant (Seq.4)

5'ATGGAGCCAGTAGATCCTAGACTAGAGCCCTGGAAGCATCCAGGAAGT  
 30 CAGCCTAAAACTGCTTGTACCAATTGCTATTGTAAAAAGTGTTGCTTTCAT  
 TGCCAAGTTTGTTCATAACAAAAGCCTTAGGCATCTCCTATGGCAGGAA

GAAGCGGAGACAGCGACGAAGACCTCCTCAAGGCAGTCAGACTCATCAA  
GTTTCTCTATCAAAGCAGCCACCTCCCAATCCCCGACAGGCCCGAAGG  
AATAG 3'

Nucleotide sequence of lys41-RGD $\Delta$  mutant (Seq.5)

5 5'ATGGAGCCAGTAGATCCTAGACTAGAGCCCTGGAAGCATCCAGGAAGT  
CAGCCTAAAACTGCTTGTACCAATTGCTATTGTAAAAAGTGTTGCTTTCAT  
TGCCAAGTTTGTTCATAACAAACGCCTTAGGCATCTCCTATGGCAGGAA  
GAAGCGGAGACAGCGACGAAGACCTCCTCAAGGCAGTCAGACTCATCAA  
GTTTCTCTATCAAAGCAGCCACCTCCCAATCCCCGACAGGCCCGAAGG  
10 AATAG 3'

10.Vaccine according to claim 6 in which mutants are selected among the ones  
having the following amino acid sequence or part of them:

Amino acid sequence of cys22 mutant

NH<sub>2</sub>-MEPVDPRLEPWKHPGSQPKTAGTNCYCKKCCFHCQVCFITKA  
15 LGISYGRKKRRQRRRPPQGSQTHQVSLSKQPTSQSRGDPTGPKE-COOH

Amino acid sequence of lys41

NH<sub>2</sub>-MEPVDPRLEPWKHPGSQPKTACTNCYCKKCCFHCQVCFITTAL  
GISYGRKKRRQRRRPPQGSQTHQVSLSKQPTSQSRGDPTGPKE-COOH

Amino acid sequence of RGD $\Delta$  mutant

20 NH<sub>2</sub>-MEPVDPRLEPWKHPGSQPKTACTNCYCKKCCFHCQVCFITKAL  
GISYGRKKRRQRRRPPQGSQTHQVSLSKQPTSQSPTGPKE-COOH

Amino acid sequence of lys41-RGD $\Delta$  mutant

NH<sub>2</sub>-MEPVDPRLEPWKHPGSQPKTACTNCYCKKCCFHCQVCFITTAL  
GISYGRKKRRQRRRPPQGSQTHQVSLSKQPTSQSPTGPKE-COOH

25 11.Vaccine according to claim 6 in which the Tat portions are selected among the  
peptide sequences

Pep. 1. MEPVDPRLEPWKHPGSQPKT

Pep. 2. ACTNCYCKKCCFHCQVCFIT

Pep. 3. QVCFITKALGISYGRK

30 Pep. 4. SYGRKKRRQRRRPPQ

Pep. 5. RPPQGSQTHQVSLSKQ

Pep. 6. HQVSLSKQPTSQSRGD

Pep. 7. PTSQSRGDPTGPKE

12. Vaccine according to claims 6-11 comprising proteins or peptides conjugated with the T-helper universal epitope of Tetanus toxoid or any T-helper peptides.
- 5 13. Vaccine according to claims 6-12 in combination with recombinant proteins or peptides of HIV Nef, Rev or Gag or part of them.
14. Vaccine according to claim 6 comprising fusion proteins Tat (wild type or its mutants)/Nef, Tat (wild type or its mutants)/Rev, Tat (wild type or its mutants)/Gag or part of them.
- 10 15. Vaccine according to claim 6-14 in combination with recombinant immuno-modulant cytokines or other molecules, or part of them, augmenting antiviral immune response.
16. Vaccine according to claim 15 in which cytokines are IL-12 and/or IL-15 or IFN $\alpha$  or IFN $\beta$ .
- 15 17. Vaccine according to claim 6 comprising fusion proteins Tat(wild type or its mutants)/immuno-modulant cytokines, Tat (wild type or its mutants)/IL-12, Tat (wild type or its mutants)/IL-15, Tat (wild type or its mutants)/other molecules, or part of them, augmenting the antiviral immune response.
18. DNA vaccine according to claims 6, 7, 9 comprising DNA encoding for Tat wild-type or its mutants or part of them, inserted in expression vectors.
- 20 19. DNA vaccine according to claims 6, 7, 9 in combination with an expression vector including HIV rev, nef and gag genes, or part of them.
20. DNA vaccine according to claims 18 or 19 in which the vector is a plasmid co-expressing tat (wild-type or its mutants)/rev, tat (wild-type or its mutants)/nef, tat (wild-type or its mutants)/gag or part of them.
- 25 21. DNA vaccine according to claims 6, 7, 9 in combination with DNA molecules inserted in expression vectors encoding for immuno-modulant cytokines or other immuno-modulant molecules, or part of them, augmenting the antiviral immune response.
- 30 22. DNA vaccine according to claim 21 in which the cytokine is IL-12 and/or IL-15.
23. DNA vaccine according to claims 21 or 22 in which the vector is a plasmid co-

expressing tat (wild-type or its mutants)/IL-12, tat (wild-type or its mutants)/IL-15, tat (wild-type or its mutants)/other molecules, or part of them, able to augment the antiviral immune response.

24. Vaccine according to claims 18-23 in which the vector is pCV0.
- 5 25. Vaccine according to the previous claims including autologous dendritic cells treated and/or untreated according to the previous claims.
26. Vaccine according to the previous claims including adjuvants able to augment the antiviral immune response.
27. Vaccine according to claim 26 in which the adjuvant is selected among Alum,  
10 ISCOM, RIBI and related mixtures.
28. Vaccine according to previous claims comprising systems for delivery.
29. Vaccine according to claim 28 in which the systems for delivery are selected among nanoparticles, herpes vectors, red cells, bacteria and combinations thereof.
- 15 30. Vaccine according to claim 29 in which bacteria are selected among *Streptococcus gordonii* and *Lactobacillus*.
31. Vaccine according to claims 29 and 30 in which bacteria are modified to express viral antigens.
32. Vaccine according to the previous claims for the immunization of peripheral  
20 blood cells from infected individuals, expanded by co-stimulation with magnetic beads coated with anti-CD3 and anti-CD28 antibodies.
33. Therapeutic vaccine according to the previous claims, combined with inhibitors of viral replication.
34. Vaccine according to claims 6-33 in which the active principle is delivered to  
25 the mucosa.
35. Vaccine according to claim 34, in which the active principle is administered nasally, orally, vaginally and/or rectally.
36. Vaccine according to claims 6-33 in which the active principle is administered through systemic or local route.
- 30 37. Vaccine according to claim 36 in which the active principle is administered through intramuscular, subcutaneous or intradermal route.

38. Vaccine according to claim 37 in which the active principle is administered intradermally at 1-6  $\mu$ g amounts, without adjuvants.

39. Vaccine according to claims 34-38 in which the active principle is carried in a biologically acceptable fluid.

5 40. Vaccine according to claims 34-39 further comprising pharmaceutically acceptable carriers and eccipients to maximize the principle activity.

41. Vaccine according to claims 34-40 comprising as active principle Tat according to claim 1 at preventive and/or therapeutic amounts.

42. Biologically active Tat nucleotide sequence (Seq. 1):

10 5'ATGGAGCCAGTAGATCCTAGACTAGAGCCCTGGAAGCATCCAGGAAGT  
CAGCCTAAACTGCTTGTACCAATTGCTATTGTAAAAAGTGTTGCTTTCAT  
TGCCAAGTTTGTTTCATAACAAAAGCCTTAGGCATCTCCTATGGCAGGAA  
GAAGCGGAGACAGCGACGAAGACCTCCTCAAGGCAGTCAGACTCATCAA  
GTTTCTCTATCAAAGCAGCCACCTCCCAATCCCGAGGGGACCCGACAG  
15 GCCCGAAGGAATAG 3'

43. Biologically active Tat amino acid sequence

NH<sub>2</sub>-EPVDPRLEPWKHPGSQPKTACTNCYCKKCCFHCQVCFITKAISY  
GRKKRRQRRRPPQGSQTHQVSLSKQPTSQSRGDPTGPKE-COOH

44. Biologically active Tat mutant protein having nucleotide sequence selected  
20 among:

Nucleotide sequence of the cys22 mutant (Seq.2)

5'ATGGAGCCAGTAGATCCTAGACTAGAGCCCTGGAAGCATCCAGGAAGT  
CAGCCTAAACTGCGGTACCAATTGCTATTGTAAAAAGTGTTGCTTTCATT  
GCCAAGTTTGTTTCATAACAAAAGCCTTAGGCATCTCCTATGGCAGGAAG  
25 AAGCGGAGACAGCGACGAAGACCTCCTCAAGGCAGTCAGACTCATCAAG  
TTTCTCTATCAAAGCAGCCACCTCCCAATCCCGAGGGGACCCGACAGG  
CCCGAAGGAATAG 3'

Nucleotide sequence of the lys41 mutant (Seq.3)

5'ATGGAGCCAGTAGATCCTAGACTAGAGCCCTGGAAGCATCCAGGAAGT  
30 CAGCCTAAACTGCTTGTACCAATTGCTATTGTAAAAAGTGTTGCTTTCAT  
TGCCAAGTTTGTTTCATAACAAACGCCTTAGGCATCTCCTATGGCAGGAA

GAAGCGGAGACAGCGACGAAGACCTCCTCAAGGCAGTCAGACTCATCAA  
GTTTCTCTATCAAAGCAGCCCACCTCCCAATCCCGAGGGGACCCGACAG  
GCCCCAAGGAATAG 3'

Nucleotide sequence of the RGD $\Delta$  mutant (Seq.4)

5 5'ATGGAGCCAGTAGATCCTAGACTAGAGCCCTGGAAGCATCCAGGAAGT  
CAGCCTAAACTGCTTGTACCAATTGCTATTGTAAAAAGTGTTGCTTTCAT  
TGCCAAGTTTGTTCATAACAAAAGCCTTAGGCATCTCCTATGGCAGGAA  
GAAGCGGAGACAGCGACGAAGACCTCCTCAAGGCAGTCAGACTCATCAA  
GTTTCTCTATCAAAGCAGCCCACCTCCCAATCCCGACAGGCCCGAAGG  
10 AATAG 3'

Nucleotide sequence of the lys41-RGD $\Delta$  mutant (Seq.5)

5'ATGGAGCCAGTAGATCCTAGACTAGAGCCCTGGAAGCATCCAGGAAGT  
CAGCCTAAACTGCTTGTACCAATTGCTATTGTAAAAAGTGTTGCTTTCAT  
TGCCAAGTTTGTTCATAACAAACGCCTTAGGCATCTCCTATGGCAGGAA  
15 GAAGCGGAGACAGCGACGAAGACCTCCTCAAGGCAGTCAGACTCATCAA  
GTTTCTCTATCAAAGCAGCCCACCTCCCAATCCCGACAGGCCCGAAGG  
AATAG 3'

45. Biologically active Tat mutants amino acid sequence selected among:

Amino acid sequence of cys22 mutant

20 NH<sub>2</sub>-MEPVDPRLEPWKHPGSQPKTAGTNCYCKKCCFHCQVCFITKA  
LGISYGRKKRRRQRRRPPQGSQTHQVSLSKQPTSQSRGDPTGPKE-COOH

Amino acid sequence of lys41 mutant

NH<sub>2</sub>-MEPVDPRLEPWKHPGSQPKTACTNCYCKKCCFHCQVCFITTAL  
GISYGRKKRRRQRRRPPQGSQTHQVSLSKQPTSQSRGDPTGPKE-COOH

25 Amino acid sequence of RGD $\Delta$  mutant

NH<sub>2</sub>-MEPVDPRLEPWKHPGSQPKTACTNCYCKKCCFHCQVCFITKAL  
GISYGRKKRRRQRRRPPQGSQTHQVSLSKQPTSQSPTGPKE-COOH

Amino acid sequence of lys41-RGD $\Delta$  mutant

30 NH<sub>2</sub>-MEPVDPRLEPWKHPGSQPKTACTNCYCKKCCFHCQVCFITTAL  
GISYGRKKRRRQRRRPPQGSQTHQVSLSKQPTSQSPTGPKE-COOH

46. Biologically active Tat mutants with peptide sequence selected among :

Pep. 1. MEPVDPRLEPWKHPGSQPKT

Pep. 2. ACTNCYCKKCCFHCQVCFIT

Pep. 3. QVCFITKALGISYGRK

5      Pep. 4. SYGRKKRRQRRRPPQ

Pep. 5. RPPQGSQTHQVSLSKQ

Pep. 6. HQVSLSKQPTSQSRGD

Pep. 7. PTSQSRGDPTGPKE

47. Expression vector comprising a DNA sequence selected among the ones listed  
10      in claims 42 and 44 or parts thereof.

48. Expression vector pCV0 including a DNA sequence selected among the ones  
listed in claims 42-44 or parts thereof.

49. Expression vector pCV0 according to claim 48, comprising a DNA sequence  
codifying for a gene selected among tat, rev, nef, gag, IL-12, IL-15 and  
15      combinations thereof.

50. Transformed cells including the vector according to claims 47-49.

51. Dendritic cells treated with Tat protein or its peptides or mutants according to  
claims 42-46 or combinations with Rev, Nef, and Gag proteins and/or cytokines.

52. Dendritic cells according to claim 51 transduced with an expression vector  
20      comprising tat gene.

53. Process for producing biologically active Tat protein or its mutants or its  
recombinant forms or parts of them comprising to cultivate the cells according  
to claim 50 and to isolate and purify the thus obtained protein or parts of it.

54. Process for preparing the pCV0 vector according to claim 49, in which the  
25      corresponding cDNA is amplified by PCR technique using primers selected  
among:

Seq. P1. Primer forward Rev: 5'ATGGCAGGAAGAAGC3'

Seq. P2. Primer reverse Rev: 5'CTATTCTTTAGTTCC3'

Seq. P3. Primer forward Nef: 5'ATGGGTGGCAAGTGG3'

30      Seq. P4. Primer reverse Nef: 5'TCAGCAGTCCTTGTA3'

Seq. P5. Primer forward Gag: 5'ATGGGTGCGAGAGCG3'



- Seq. P6. Primer reverse Gag: 5'TTATTGTGACGAGGG3'
- Seq. P7. Primer forward IL-12: 5'ATGTGGCCCCCTGGG3'
- Seq. P8. Primer reverse IL-12: 5'TTAGGAAGCATTTCAG3'
- Seq. P9. Primer forward IL-15: 5'ATGAGAATTTTCGAAA3'
- 5 Seq. P10. Primer reverse IL-15: 5'TCAAGAAGTGTTGAT3'
- Seq. P11. Primer forward Tat: 5'ATGGAGCCAGTAGAT3'
- Seq. P12. Primer reverse Tat: 5'CTATTCCTTCGGGCC3'
- Seq. P13. Primer forward Tat/Rev: 5'GGCCCGAAGGAAATGGCA  
GGAAGAAGC3'
- 10 Seq. P14. Primer forward Tat/Nef: 5' GGCCCGAAGGAAATGGGT  
GGCAAGTGG3'
- Seq. P15. Primer forward Tat/Gag: 5' GGCCCGAAGGAAATGGGT  
GCGAGAGCG3'
- Seq. P16. Primer forward Tat/IL-12: 5' GGCCCGAAGGAAATGTGGC  
15 CCCCTGGG3'
- Seq. P17. Primer forward Tat/IL-15: 5' GGCCCGAAGGAAATGAGAAT  
TTCGAAA3'
55. Primer selected among:
- Seq. P1. Primer forward Rev: 5'ATGGCAGGAAGAAGC3'
- 20 Seq. P2. Primer reverse Rev: 5'CTATTCTTTAGTTCC3'
- Seq. P3. Primer forward Nef: 5'ATGGGTGGCAAGTGG3'
- Seq. P4. Primer reverse Nef: 5'TCAGCAGTCCTTGTA3'
- Seq. P5. Primer forward Gag: 5'ATGGGTGCGAGAGCG3'
- Seq. P6. Primer reverse Gag: 5'TTATTGTGACGAGGG3'
- 25 Seq. P7. Primer forward IL-12: 5'ATGTGGCCCCCTGGG3'
- Seq. P8. Primer reverse IL-12: 5'TTAGGAAGCATTTCAG3'
- Seq. P9. Primer forward IL-15: 5'ATGAGAATTTTCGAAA3'
- Seq. P10. Primer reverse IL-15: 5'TCAAGAAGTGTTGAT3'
- Seq. P11. Primer forward Tat: 5'ATGGAGCCAGTAGAT3'
- 30 Seq. P12. Primer reverse Tat: 5'CTATTCCTTCGGGCC3'
- Seq. P13. Primer forward Tat/Rev: 5'GGCCCGAAGGAAATGGCA

GGAAGAAGC3'

Seq. P14. Primer forward Tat/Nef: 5' GGCCCGAAGGAAATGGGT  
GGCAAGTGG3'

Seq. P15. Primer forward Tat/Gag: 5' GGCCCGAAGGAAATGGGT  
5 GCGAGAGCG3'

Seq. P16. Primer forward Tat/IL-12: 5' GGCCCGAAGGAAATGTGGC  
CCCCTGGG3'

Seq. P17. Primer forward Tat/IL-15: 5' GGCCCGAAGGAAATGAGAAT  
TTCGAAA3'.

- 10 56.Process for preparing a vaccine according to claims 6-41, wherein Tat is in its non oxidated form.
- 57.Process for preparing a vaccine according to claims 6-41, wherein Tat, in its lyophilized form, is re-suspended in a biologically acceptable fluid for administration.
- 15 58.Use of Tat protein wild-type in its active form and/or its mutants and/or parts related to the protein or peptides or the DNA encoding for these proteins or parts of them or peptides to make a protein or peptide or DNA vaccine, preventive and/or therapeutic, against AIDS, tumors, the syndromes and symptoms associated to HIV infection.
- 20 59.Use of Alum, ISCOM, RIBI and other adjuvants, alone or in combination, to make a vaccine according to claim 6.
- 60.Use of paramagnetic beads coated with monoclonal antibodies anti-CD3 and anti-CD28 to make a vaccine according to claim 6.
- 25 61.Therapeutic method for treating AIDS, tumors, syndromes and symptoms associated with HIV infection characterized in that preventive or therapeutic amounts of biologically active Tat according to claims 1-5 are administered.

HIV-1 Tat, or derivatives thereof, alone or in combination, for prophylactic and therapeutic vaccination against AIDS, tumors and related syndromes

**Abstract**

The present invention refers to Tat as the active principle for a prophylactic and/or  
5 therapeutic vaccine against HIV infection, the progression towards AIDS and the  
development of tumors and other syndromes and symptoms in subjects infected by  
HIV. Tat is in biologically active form either as recombinant protein or peptide or as  
DNA. More particularly, the invention refers to a vaccine based on HIV-1 Tat as  
immunogen, inoculated as DNA and/or recombinant protein or as peptides, alone or  
10 in combination with other genes or viral gene products (Nef, Rev, Gag) or parts  
thereof, or in combination with various immuno modulant cytokines (IL-12, IL-15) or  
with the gene coding for an immuno modulant cytokine or part thereof. Tat, Nef, Rev,  
Gag and the immuno modulant cytokines are administrated both as a mixture of  
recombinant proteins, peptides or fusion proteins (Tat/Nef, Tat/Rev, Tat/Gag, Tat/IL-  
15 12, Tat/IL-15) or as plasmid DNA.